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Opportunities for organoids as new models of aging

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The biology of aging is challenging to study, particularly in humans. As a result, model organisms are used to approximate the physiological context of aging in humans. However, the best model organisms remain expensive and time-consuming to use. More importantly, they may not reflect directly on the process of aging in people. Human cell culture provides an alternative, but many functional signs of aging occur at the level of tissues rather than cells and are therefore not readily apparent in traditional cell culture models. Organoids have the potential to effectively balance between the strengths and weaknesses of traditional models of aging. They have sufficient complexity to capture relevant signs of aging at the molecular, cellular, and tissue levels, while presenting an experimentally tractable alternative to animal studies. Organoid systems have been developed to model many human tissues and diseases. Here we provide a perspective on the potential for organoids to serve as models for aging and describe how current organoid techniques could be applied to aging research.

Introduction

Aging is widely held to be the consequence of the accumulation of cellular damage over time (López-Otín et al., 2013). In humans, aging is marked by declining tissue function and increased susceptibility to diseases such as cancer, diabetes, cardiovascular disease, dementia, arthritis, sarcopenia, and renal dysfunction. Aging-associated diseases and phenotypes frequently manifest on the tissue level yet have their roots in molecular and cellular damage (López-Otín et al., 2013).

Most aging research is motivated by the need to understand the process of aging in humans. However, studying aging requires observing and perturbing molecules, cells, tissues, and systems in organisms over time—requirements that are not feasible in humans. Instead, aging is studied in model systems, each of which balances a desire for biological relevance on one hand and practicality on the other. Thus, most models of aging suffer from key limitations depending on where on this spectrum they lie. Organoid culture, especially human organoid culture, has the potential to address many of the limitations of traditional aging models. By providing organotypic structure in an in vitro model, organoids enable the study of aging in

tissues in a higher-throughput, experimentally accessible manner. These potential advantages may be particularly useful for studying emergent signs of aging at the tissue level, clearing the way for future human therapies.

Models of aging

The spectrum of aging models. The trade-off between relevance to humans and ease of experimentation is a limitation in any model, particularly when seeking to develop interventions to limit the effects of human aging. Organoids provide a powerful and physiologically relevant means to perform in vitro experiments on tissue and on human tissue in particular.

Nonhuman organisms and traditional cell culture have been invaluable tools for the study of fundamental aging processes, including the Nobel Prize-winning mechanisms of telomere regulation and DNA repair (Sancar and Rupp, 1983; Greider and Blackburn, 1987; Lindahl, 1993). However, model organisms may age in ways not applicable to humans. For example, *Caenorhabditis elegans* has a dauer stage, a stress- and aging-resistant larval form. Induction of dauer-like states may alter lifespan with questionable correlation to human aging (McElwee et al., 2006). In mice, the most common mammalian model organism, important features that affect aging, such as immune response, DNA repair, telomerase regulation, and telomere length, differ significantly between mice and humans (Rangarajan and Weinberg, 2003; Seok et al., 2013). Even naked mole rats and blind mole rats, two rodents both renowned for their sevenfold-longer lives than laboratory mice, owe their longevity to differing molecular mechanisms (Azpúrua and Seluanov, 2013). Thus, different species, even within the same order, have developed distinct mechanisms for controlling lifespan and health span in relatively brief evolutionary time. These differences have practical implications. Some of the most prominent diseases of human aging, such as epithelial cancers, cardiovascular disease, osteoporosis, and Alzheimer's disease, are rare or nonexistent in aged mice, who instead predominantly suffer from mesenchymal cancers (Rangarajan and Weinberg, 2003; Vanhooren and Libert, 2013). Consequently, interventions that reduce susceptibility to human age-related diseases may not affect mouse lifespan, and vice versa.

Ultimately, studies in humans are necessary to validate therapies for human aging, but ethical, logistical, and financial challenges render routine human aging studies infeasible. A widely practiced alternative is cell culture, but the correlations

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What is an organoid?

An organoid is a miniature, simplified organ that recreates physiological 3D tissue structure and cellular composition *in vitro*. Examples include a variety of 3D *in vitro* culture systems: cultured tissue fragments, tissues reconstituted from cultured cells, and tissues grown from stem cells, usually in the presence of ECM protein. In this perspective, we have broadly included examples and influences from several different organotypic culture systems. The use of organoids reflects the philosophy that the structure and composition of a tissue is relevant to its function (Simian and Bissell, 2017). In practice, organoids are characterized by some if not all of the following features:

- A physiologically relevant microenvironment
- Cells derived from primary tissues, primary cells, or stem cells, rather than immortalized cell lines
- Cells correctly organized with respect to one another and their ECM
- Multiple cell types from the original organ

to human aging may be weak because of the lack of physiological context. Many signs of aging, namely those that manifest at the tissue and organ level, may be best represented by a more complex system. These signs of aging include a breakdown in tissue homeostasis, stem cell dysfunction, and susceptibility to tissue-specific diseases and cancer (López-Otín et al., 2013).

Although more technically challenging than traditional cell culture, organoids provide increased relevance to human health via a microenvironment supportive of tissue-specific function and physiological cell–cell and cell–matrix interactions. These factors contribute to increased viability of difficult-to-culture cells, including primary cells and prostate tumors (Gao et al., 2014). Moreover, 3D culture improves cell type-specific function and gene expression in cells such as chondrocytes, hepatocytes, and mammary and kidney epithelial cells (Ben-Ze'ev et al., 1988; Astashkina et al., 2012; Caron et al., 2012; Simian and Bissell, 2017). As a result, organoids may be useful for modeling age-related declines in tissue-specific function, such as chondrocyte matrix deposition (Barbero et al., 2004). Furthermore, age-related pathologies intrinsically linked to the ECM, such as osteoarthritis, are well-suited to study in a 3D system (Lozito et al., 2013). Organoids may be the most practical way to perform aging experiments at this level of complexity, particularly on human tissues.

Practical experiments with organoid models.

Organoids provide a practical alternative to whole-organism studies in mammals. Patient-derived organoids have been scaled to high-throughput screens against cystic fibrosis and cancer, which would be cost-prohibitive in mice or humans (Dekkers et al., 2016; Pauli et al., 2017; Schütte et al., 2017). Demonstrating the scalability and high-throughput potential of organoids, Pauli et al. (2017) developed patient-derived tumor organoids for screening a library of >100 drugs at different concentrations and combinations, and Danahay et al. (2015) screened nearly 5,000 proteins for their effects on basal cell fate in human lung organoids.

Improved throughput is made possible not only by larger sample sizes but also simpler assays. Some molecular signs of aging, such as protein aggregation, oxidation, and glycation, can be assayed by nondestructive, high-throughput microscopy (Meerwaldt et al., 2004; Chaudhuri et al., 2006; Jung et al., 2010). Organoids are far more amenable to live-cell imaging than whole organisms, such as mice, and can complement mouse studies with imaging and perturbations that would be otherwise impractical. High-resolution live imaging of

development, stem cell dynamics, and cancer invasion has been demonstrated in several organoid systems (Sato et al., 2011; Nguyen-Ngoc et al., 2015).

Finally, manipulating microenvironmental cues and specific cell populations is more straightforward in organoids than model organisms. Organoids are typically embedded in a matrix of the investigators' choice, allowing for increased control over its source, composition, and mechanical properties. Cells acquired from tissue can be sorted, modified to delete or express specific genes, and expanded before assembly into organoids, allowing investigators to build organoids using defined building blocks from the bottom up. These organoids enable otherwise infeasible studies, such as tissues bearing gene knockouts that would be embryonically lethal. Additionally, this strategy allows the construction of organoids composed of specific proportions of cell subpopulations. For example, Sato et al. (2011) assembled intestinal organoids from stem cells with or without Paneth cells to identify Paneth cells as essential components of the intestinal stem cell (ISC) niche, a result previously obscured by incomplete lineage ablation. Moreover, viral vectors or transfection allows genetic manipulation of organoids with far less time and expense than developing a transgenic mouse (Koo et al., 2011). As a result, organoid approaches greatly enhance our capacity to study specific cell populations, phenotypes, and mutations within the context of a tissue. Organoid models of aging are in their earliest stages, but promising work, discussed in this perspective, has demonstrated their potential applicability to aging research.

Studying aging in organoids

Molecular and cellular processes. The genome can be damaged through several mechanisms, leading to the acquisition of genetic and epigenetic changes with age. DNA damage activates repair pathways but can also trigger mutations, apoptosis, or senescence. These outcomes can lead to aberrant behavior and deleterious effects on neighboring cells, such as inflammatory senescence-associated signaling (Parrinello et al., 2005). Epigenetically, aging cells acquire regional hyper- and hypomethylation, as well as increased variance in epigenetic marks (Jones et al., 2015). These cell-intrinsic aspects of aging are preserved in primary cells and tissue-derived organoids (Blokzijl et al., 2016).

Glycation, oxidation, and misfolding are foremost among molecular damage to proteins during aging. In particular, ECM proteins such as collagen, elastin, and crystallin are susceptible to nonenzymatic glycation, leading to stiffening of the ECM (Singh et al., 2001). Age-associated glycation, as well as altered structure, composition, and cross-linking of the ECM, has been associated with functional declines in a variety of tissues (Phillip et al., 2015). As matrix is a major component of organoid culture, organoids are a natural model for the effects of aged ECM on tissue function.

Organoid assays can enable researchers to experimentally link aging-associated changes in ECM structure to functional consequences. ECM substrates and gels for cell culture have been isolated from human and animal tissue (Voytik-Harbin et al., 2007; Zhang et al., 2009; O'Brien et al., 2010). Decreased cell proliferation and stem cell function have been observed on matrices derived from older donors, including Bruch's membrane and cardiac and skeletal muscle ECM (Gullapalli et al., 2005; Williams et al., 2014; Stearns-Reider et al., 2017). More complex phenotypes can also be linked to ECM source by using

organoid models. The Schedin group (Lyons et al., 2011) has shown that ECM from rats in different reproductive states can instruct morphogenesis and invasion of human mammary and breast cancer organoids. In addition, several groups have experimentally modified the ECM via advanced glycation end product-mediated cross-linking to mimic ECM aging, demonstrating aging phenotypes such as skin and cartilage stiffening and increased invasiveness (Verzijl et al., 2002; Pigeon and Asseineau, 2005; Rodriguez-Teja et al., 2015).

Chemical damage can also lead to degradation and aggregation of proteins and other biomolecules, such as lipids. Protein damage accumulates in the form of carbonylation, fragmentation, and cross-linking, whereas lipid peroxidation compromises membranes and leads to the accumulation of oxidized fatty acids. Oxidation products not only have reduced function but are also prone to cross-linking. Together with misfolding, age-associated protein oxidation results in proteolysis-resistant aggregates both intra- and extracellularly. This feature is highly conserved across species; one of the most universal signs of aging is the accumulation of intracellular lipofuscin lipid-protein aggregates (Jung et al., 2010). Although their causal role in pathogenesis is disputed, protein and lipid-protein aggregates are prominently associated with central nervous system dysfunction, such as the infamous tangles, amyloids, and Lewy bodies in neurodegenerative disease, and the retinal drusen in age-related macular degeneration (Crabb et al., 2002; Caughey and Lansbury, 2003). These late-stage manifestations of neurodegenerative pathology may be more apparent in 3D culture than in traditional cell culture, where secreted proteins diffuse freely into culture media rather than accumulate near cells. In a human stem cell-derived model of familial Alzheimer's disease, 3D culture was found to not only improve neuronal maturation but also promote tauopathy through the accumulation of β -amyloid aggregates in the ECM, observations that did not occur in 2D culture or mice (Choi et al., 2014). Similarly, scaffold-free 3D neural organoids, but not 2D culture, were shown to accumulate amyloid aggregates and model tauopathy. Significantly, these signs of human neurodegeneration were spontaneous in patient-derived organoids but are often only observed in mice after the expression of multiple human transgenes (Raja et al., 2016).

Tissue-level processes. At the tissue level, accumulated molecular damage can manifest in a variety of ways. These include changes in tissue function, susceptibility to cancer and other diseases, changes in stem cell function, and alterations in the immune system (Aw et al., 2007; López-Otín et al., 2013; Blau et al., 2015). Encouragingly, a variety of organoid models have already been explored to study aspects of each of these age-related phenomena.

One feature of aging is a progressive decline in physiological function. Various signs of aging, such as lower glomerular filtration rate, reduced cardiac output, and increased susceptibility to disease, can often be tied to tissue-specific changes that may be more apparent in 3D tissues than 2D culture. For example, a semi-enclosed cardiac organoid chamber allows for the measurement of pressure and ejection fraction (Lee et al., 2008). Barrier function can be measured across a kidney- or neural-endothelial interface (Verneti et al., 2017), which could be used to model aging-associated declines in glomerular filtration rate and blood-brain barrier function (Tan et al., 2010; Elahy et al., 2015). Additionally, gastrointestinal organoids have been used extensively to study host-microbe interactions (Bartfeld et

al., 2015; Engevik et al., 2015). They would be well-suited to studying the aging microbiome and age-related susceptibility to disease caused by ordinarily asymptomatic microbes, such as *Clostridium difficile* (Htwe et al., 2007; Heintz and Mair, 2014). Age-associated changes in circadian rhythm may also be modeled in organoids, considering that intestinal explants and stem cell-derived organoids, but not 2D culture, have been demonstrated to spontaneously produce robust, synchronized circadian oscillations (Myers and Badia, 1995; Moore et al., 2014). In general, organotypic cultures can represent more complex and accurate phenotypes than 2D culture and are thus ideal for modeling their decline in aging.

Organoids are widespread models for cancer progression that have been used to study tumor-initiating mutations and processes, such as cancer cell dissemination (Li et al., 2014; Nguyen-Ngoc et al., 2015). A natural extension of this work would be to use organoids from donors of different ages to understand how normal, aged tissue responds to in vitro oncogenic transformation. For example, transformed human mammary epithelial cells develop phenotypes dependent on donor age. Aged cells more frequently adopted a luminal-like phenotype and expressed estrogen receptor α in organoid culture, suggesting that age is a determinant of cancer subtype (Lee et al., 2015). In addition, organoid culture permits study of the tumor microenvironment, including neighboring cells and the ECM. Age-related changes to the microenvironment may play a role in altered susceptibility to cancer. Experimental glycation of the ECM increased invasiveness and decreased epithelial differentiation in nonmalignant prostate acini (Rodriguez-Teja et al., 2015). Similarly, the accumulation of senescent cells with age may exert tumor-promoting effects on neighboring cells. By coculturing fibroblasts with nonmalignant mammary organoids, Parrinello et al. (2005) demonstrated that senescent fibroblasts secreted factors that increased invasion and proliferation, and decreased epithelial differentiation.

Organoids are excellent models for stem cell maintenance and tissue repair, because organoids can recreate in vitro the minimal unit of a stem cell niche: cells and ECM in an organotypic structure (Murrow et al., 2017). Stem cell exhaustion, or reduced proliferation and biased differentiation of stem cells, contributes to aging in several tissues, and stem cell function is a common readout in aging studies (Brack et al., 2007; Pang et al., 2011; Garbe et al., 2012). For example, mouse intestinal organoids have been used to study the proliferation and differentiation of ISCs in the context of aging research. In one study of ISC function, which declines with age, Nalapareddy et al. (2017) used organoids from aged mice to determine that the aged ISC niche was deficient in Wnt signaling. Exogenous Wnt3a supplementation in organoid culture was sufficient to restore organoid formation and lobe growth of aged ISCs. Other investigators have used organoid-formation assays to study caloric restriction, an intervention shown to have anti-aging effects in several model organisms, including nonhuman primates (Mattison et al., 2017). By combining the Paneth cells and ISCs of ad libitum-fed and calorie-restricted mice, Yilmaz et al. (2012) demonstrated that Paneth cells from calorie-restricted mice were responsible for increasing ISC organoid formation and size. The capacity to separate and perturb the contributions of individual niche components, such as growth factors and accessory cells, during aging and anti-aging interventions in a rapid, systematic manner is an important advantage that organoids provide over model organisms.

Aging of the immune system is characterized by changing proportions of immune cells, declining innate immune function, and increased chronic inflammation (Aw et al., 2007). To study how these factors intersect with age-associated diseases such as cancer and osteoarthritis, aging researchers can take advantage of existing methods for studying the effects of immune cell co-culture and pro-inflammatory stimuli in organoids. For example, co-culture of primary mouse mammary epithelial organoids and immune cells demonstrated how immune cells regulate branching morphogenesis (Plaks et al., 2015). Systemic inflammation has also been studied in organoids by adding inflammatory cytokines or antiinflammatory drugs to the culture medium. Anti-inflammatory treatment of intestinal organoids suppressed the senescence-associated inflammatory response and reduced inflammation-mediated tumorigenesis (Pribluda et al., 2013). Sun et al. (2011) used both cytokines and macrophage-conditioned media to simulate immune involvement in a 3D human chondrocyte model of osteoarthritis. In response to inflammation, the cartilage scaffolds demonstrated phenotypic features of early osteoarthritis, such as increased and atypical matrix synthesis and degradation. These examples highlight systems that integrate organoids with immune cells and inflammatory cytokines.

Sourcing organoids

Organoids can be derived from several sources, most notably primary cells and tissue or pluripotent stem cells (PSCs). Cell lines are less suitable sources for most applications of organoids to aging research, because of immortalization, evasion of cellular senescence, or tumor origins. In contrast, tissues from donors of different ages are a more suitable model of the phenotypes of aging. These samples are becoming more readily available to the basic science research community. A handful of untransformed primary human cell types and tissues, such as keratinocytes, stromal and dermal fibroblasts, and mammary epithelial cells, are available from cell banks. Skin, blood, marrow, fat, breast, and muscle are relatively abundant tissues that can be acquired from healthy donors undergoing elective surgery. In addition, primary human material can be obtained from pathologically normal tumor-peripheral tissue, surgical discards, tissues ineligible for transplant, and trimmings from transplanted tissues (Barkauskas et al., 2017).

Fresh, primary tissue explants or fragments can be cultured with minimal processing, often retaining multiple cell types and the surrounding donor-derived ECM and stroma (Fig. 1 A; Tanos et al., 2013; Nguyen-Ngoc et al., 2015; Li et al., 2016). Some, such as arteries, skin, and central nervous system, are typically cultured as thin slices; alternatively, organoids may be derived from fragments of digested minced tissue. Both strategies maintain original tissue structure, minimize disruption to cell state, and may include ECM and accessory cells. The maintenance of intact paracrine signaling networks contributes to the overall function of tissue fragment-derived organoids. For example, the stromal compartment in intestinal organoids can maintain the stem cell niche without exogenous growth factors (Ootani et al., 2009). Similarly, human mammary organoids retaining stromal and ECM elements maintain hormone responsiveness and proliferate without exogenous growth factors (Tanos et al., 2013). These organoids represent the most physiologically relevant *in vitro* models and can be particularly valuable for studying complex, intercellular signaling networks in aging tissue. However, sample size and tissue

types are limited, and it can be difficult to specifically perturb one component or cell type in the system.

In contrast, primary human cells can be dissociated, optionally expanded in culture, and then reconstituted into organoids. These reconstituted organoids allow for expanded cell numbers and considerably more control over constituent cells and ECM, including the opportunity to perturb specific components (Fig. 1 B). Although tissue composition and cell state change rapidly on 2D culture, suitable conditions for preserving lineage-specific markers during expansion have been identified in a variety of tissues, including keratinocytes, fibroblasts, and mammary and lung epithelial cells (Wilson et al., 1992; Stampfer et al., 2013; Barkauskas et al., 2017). Lung epithelial cells can be expanded in culture and give rise to branched bronchial or alveolar organoids (Barkauskas et al., 2017). Human mammary epithelial cells are a notable aging resource established from surgical discards of donors of different ages and disease status, including healthy women and cancer patients (Stampfer et al., 2013). Nonimmortal mammary epithelial cells can be expanded *in vitro* for several passages, retain transcriptional signatures of their source tissue, and recapitulate the bilayered structure of the mammary gland in reconstituted organoids (Garbe et al., 2012; Cerchiari et al., 2015). One caveat is that expansion in culture selects for proliferative cells, potentially excluding cells with short telomeres, terminally differentiated nonproliferating cells, and cells undergoing senescence. Furthermore, 2D culture also causes loss of cell type-specific transcriptional profiles; some of this dedifferentiation can be reversed or ameliorated on transitioning to organotypic culture (Caron et al., 2012).

Primary tissue is also a source of multipotent adult tissue stem cells that can generate organoids in the presence of specific growth factors; stem cell-derived organoids are also generated from PSCs (Fig. 1 C). Organoids from multiple epithelial tissues, including stomach, small intestine, colon, liver, pancreas, breast, and prostate, have been expanded from single primary stem or progenitor cells (Table 1). However, an issue with this method is that their maintenance requires the inclusion of stem cell niche-maintaining soluble factors, which may obscure phenotypes of aging that originate in growth factor signaling (Broutier et al., 2016; Nalapareddy et al., 2017). In contrast, PSCs, including induced PSCs (iPSCs) and embryonic stem cells allow for a greater range of key human tissue types than can be practicably supplied by surgical discards, notably brain and cardiac muscle. These organoids frequently contain multiple cell types and self-organizing tissue structures that recapitulate embryonic development. For example, human iPSC-derived brain organoids have been used to model early stage and developmental disorders (Qian et al., 2016) and could become excellent models for neurodegenerative diseases such as Alzheimer's disease. Raja et al. (2016) demonstrated that iPSC organoids derived from patients with familial Alzheimer's disease develop robust pathology, including amyloid aggregation, hyperphosphorylated tau protein, and endosome abnormalities. Caution must be exercised when applying stem cell studies to aging research. Although familial disease-linked mutations are widely studied, genetic disease may differ mechanistically from sporadic, age-related disease. Furthermore, PSCs begin in a fetal-like state and require additional maturation. Many hallmarks of aging, aside from somatic mutations, are reverted on iPSC reprogramming, including DNA damage foci, epigenetic marks, oxidative damage, senescence markers, and telomere length (Horvath, 2013; Cornacchia and Studer, 2017).

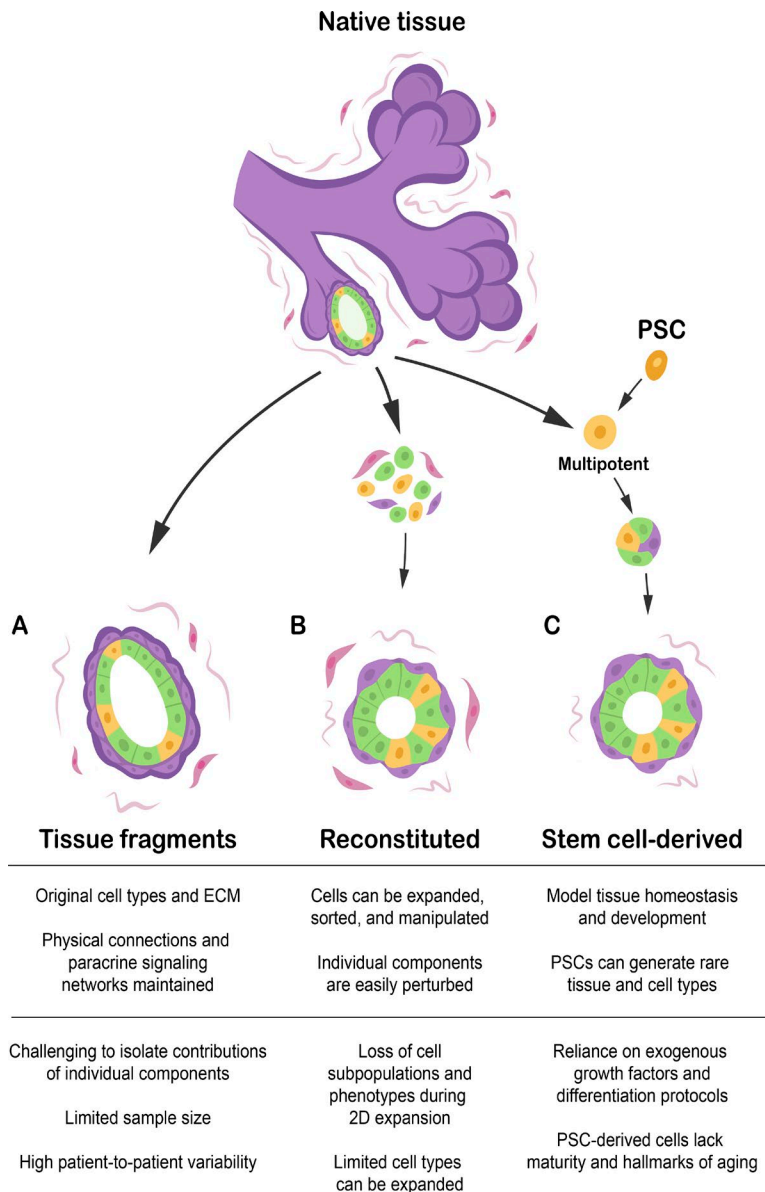


Figure 1. Types of organoids. (A) Tissue fragments and explants are generated from native tissue with little disruption to cell and ECM organization and no intermediate period in 2D culture. This limits total sample size and manipulability but is unmatched in mimicking the original tissue. (B) Reconstituted organoids are assembled in vitro from cultured or sorted cells. This permits cell expansion and modular assembly of components. However, some subpopulations or phenotypes, such as senescent and hormone-responsive cells, may be lost during culture. (C) Stem cell-derived organoids are differentiated from multipotent adult tissue stem cells or PSCs under the appropriate differentiation and maintenance conditions. PSCs specifically can generate rare tissue and cell types, but have limited maturity and lose the hallmarks of aging.

Outlook

Advances in organotypic culture techniques ultimately seek to obtain relevant phenotypes in vitro. Remarkable progress has been made in developing organoid techniques and culture systems, but several limitations remain to be addressed or validated. Furthermore, there are many opportunities to improve on the reproducibility and complexity of organoids, particularly by the inclusion of tissue engineering and microfluidics. These areas of current research are summarized in Table 2.

Although pluripotent stem cells are a significant source for organoids, one concern is the immaturity of cells and tissues derived from these cells, because PSCs begin in a fetal-like state. Functional and developmental maturation of stem cells is an active area of research and has been pursued by techniques such as long-term culture, altered concentrations of growth factors and inhibitors, direct differentiation, and mechanical or electrical stimulation (Chambers et al., 2012; Nicholas et al., 2013; Mertens et al., 2015; Ruan et al., 2016; Takasato et al., 2016; Tzatzalos et al., 2016). In vivo maturation, typically by implantation into a mouse, has also been demonstrated to

increase organoid maturity and can be used to generate adult-like human tissue and organoids (Huch et al., 2013; Watson et al., 2014; Takebe et al., 2015).

Similarly, accelerated aging in vitro will be necessary for PSC-derived organoids and useful for studying the functional consequences of age-associated damage. Accelerated aging experiments typically involve cells derived from progeric individuals (Liu et al., 2011; Miller et al., 2013) or expose cells to aging-associated stress, such as reactive oxygen species, inflammation, or radiation, which appear to phenocopy aspects of aging (Nasto et al., 2013; Cornacchia and Studer, 2017). With regard to aging experiments, organoid culture, as opposed to cell culture, has the advantage of increased stability. This stability has allowed culture periods as long as 60 wk (Hibiya et al., 2017), which can be used to study chronological aging in vitro. For example, 120 d of culture of reconstituted human epidermis led to increased cellular senescence and morphological changes resembling those of chronological skin aging in vivo (Dos Santos et al., 2015). Furthermore, genomic stability has been reported to be higher in organoids than in traditional cell

culture, as demonstrated by the use of organoid culture to clonally expand stem cells before mutational analysis (Behjati et al., 2014; Huch et al., 2015; Blokzijl et al., 2016). Notably, organoid clonal expansion was also used to quantify the accumulation of mutations in adult ISCs with age (Blokzijl et al., 2016).

Primary cell and organoid culture remains highly sensitive to culture conditions. Support of the ISC niche, for example, was a revolutionary advance, permitting long-term expansion of intestinal organoids that once could be cultured for only a couple of weeks (Ootani et al., 2009). Other tissues, such as artery or ovary, can still only be cultured for only short times. Additionally, aging studies may be particularly sensitive to culture conditions. Numerous culture parameters that can phenocopy aspects of aging, such as replicative aging, oxygen tension, and free glucose, must be carefully controlled for. Finally, long-term culture and studying tissue homeostasis require the maintenance of growth-arrested organoids resembling the resting state of adult tissues. Identifying microenvironmental conditions to transition organoid tissues from a proliferative to a more mature, growth-arrested state would represent an important contribution to aging research.

As *in vitro* models increasingly seek to recapitulate complex tissue- and organ-level phenotypes, transport of oxygen and nutrients by diffusion becomes limiting. Air–liquid interfaces have been used to culture larger explants, but in general, vasculature will be necessary for the survival of most organoids over a couple hundred micrometers wide (Auger et al., 2013), as well as useful for the study of aging-relevant phenotypes, such as metastasis and deterioration of the blood–brain barrier and glomerulus (Tan et al., 2010; Elahy et al., 2015).

Furthermore, vascularization has the potential to network organoids into systemic, multiorgan contexts that allow the exchange of hormones, cytokines, and metabolites, which have been implicated in aging in mice (Villeda et al., 2014). Incorporation into a systemic environment presents an opportunity to complement the strengths of organoid and *in vivo* platforms: organoids generated and manipulated *in vitro* can be implanted into mice, where they mature and integrate into the host vasculature (Watson et al., 2014; Takebe et al., 2015). Such systems could be powerful tools to study the effects of an aged systemic environment on human tissues.

Similarly, microfluidics could increase the complexity of *in vitro* organoid systems while improving throughput and reproducibility. Multiorgan systems have been developed for the study of organ interactions via an *in vivo*-like sequential, organ-to-organ transfer of media, with a particular interest in drug interactions (Verneti et al., 2017). These devices could provide insight into how aging tissues affect other parts of the body. Additionally, microfluidic devices permit lumen access and control over millimeter-scale morphology. Although complex tissue structures, such as intestinal crypts and kidney nephrons, are readily generated by organoid self-organization (Morizane et al., 2015), the process can be highly heterogeneous, making assay readout complex or impossible. For example, reproducible kidney organoids with a channel-accessible central duct would enable key functional assays, namely permeability and urea concentration. Methods for micron-scale organization of cells within a tissue or microfluidic chamber (Nelson et al., 2008; Todhunter et al., 2015; Kolesky et al., 2016) could be used to create reproducible, high-throughput organoid assays for aging and other diseases.

Table 1. Examples of rodent and human tissues cultured as organoids

Tissue	Type			Examples
	Tissue fragments	Reconstituted	Stem cell derived	
Central nervous system: brain, retina	Human, rat	Rat	hiPSC, hESC	Xiang et al., 2000; van Vliet et al., 2007; Hansen et al., 2010; Eiraku et al., 2011; Qian et al., 2016; Matsui et al., 2017
Gastrointestinal: esophagus, stomach, small intestine, colon	Human, mouse		Human, mouse, hPSC, mESC	Ootani et al., 2009; Barker et al., 2010; Sato et al., 2011; Bartfeld et al., 2015; Noguchi et al., 2015; Li et al., 2016
Pancreas	Human, mouse		Human, mouse, hiPSC	Huch et al., 2013; Marciniak et al., 2014; Broutier et al., 2016; Hohwieler et al., 2017
Liver	Human, mouse	Human	Human, mouse, hiPSC	Messner et al., 2013; Huch et al., 2015; Takebe et al., 2015; Broutier et al., 2016
Kidney	Mouse	Human, mouse	hiPSC, hESC	Astashkina et al., 2012; Chang and Davies, 2012; Freedman et al., 2015; Morizane et al., 2015
Lung	Human, mouse	Human, mouse	hiPSC, mouse	Moore et al., 2005; Wu et al., 2010; Barkauskas et al., 2013; Dye et al., 2015; Hegab et al., 2015
Muscle: skeletal, cardiac		Human, rat, mouse	hiPSC	Lee et al., 2008; Madden et al., 2015; Tzatzalos et al., 2016
Skin	Human	Human		Stojadinovic et al., 2013; Dos Santos et al., 2015
Cartilage	Human	Human	hMSC	Squires et al., 2003; Caron et al., 2012; Lozito et al., 2013; Bhuniratana et al., 2014
Artery	Rat	Human, mouse		Belmin et al., 1995; L'Heureux et al., 1998
Mammary gland	Human, mouse	Human	Human	Villadsen et al., 2007; Linnemann et al., 2011; Tanos et al., 2013; Nguyen-Ngoc et al., 2015
Prostate	Human	Human, rat	Human, mouse	Nevalainen et al., 1993; Lang et al., 2001; Karthaus et al., 2014
Ovary, endometrium	Human	Human		Kwong et al., 2009; Turco et al., 2017
Thyroid	Human		mESC	Toda et al., 2002; Antonica et al., 2012

Organoid culture has now been extended to a variety of tissues, including many from adult human primary tissue or cells. h, human; m, mouse; MSC, mesenchymal stem cell.

Organoids are a powerful emerging tool for the study of human health and disease. Aging research has long needed a model with the practical advantages of simpler organisms and traditional cell culture, as well as the complexity required to model the tissue-level phenotypes that accompany aging. As

researchers develop organotypic cultures of increasing complexity, it is becoming clear that organoids will enable the study of tissue-level, and even systems-level, phenotypes of aging. Human organoids have the potential to model the human body with unmatched fidelity. With time, new models will be

Table 2. Summary of current limitations and areas for development in organoid culture for aging

Goals	Approaches	Challenges
Increase developmental maturity of pluripotent stem cell-derived organoids	Long-term culture (Nicholas et al., 2013; Takasato et al., 2016; Tzatzalos et al., 2016) In vivo maturation (Huch et al., 2013; Watson et al., 2014; Takebe et al., 2015) Mechanical or electrical conditioning of muscle and cartilage (Ruan et al., 2016) Improved culture conditions and differentiation protocols Acceleration by small molecules (Chambers et al., 2012)	Stem cells are highly sensitive to culture conditions, impeding robust protocols
Source culturable, age-varied human cells	Surgical discards from elective surgery, transplant trimmings, and tissue peripheral to tumors	Hard to source certain tissues, especially healthy, culturable adult cells Cells adapt to culture and are not infinitely renewable Nontumor peripheral tissue may differ from healthy tissue
In vitro aging to model the effects of specific age-related lesions and provide a source of artificially aged cells	Induced senescence by DNA damage or environmental stress (Busuttil et al., 2003; Parrinello et al., 2005; Nasto et al., 2013) Long-term culture (Dos Santos et al., 2015) Progeria mutations (Liu et al., 2011; Zhang et al., 2011; Miller et al., 2013) Direct reprogramming of aged cells (Mertens et al., 2015)	Controversial which treatments best phenocopy aging
Mimic the effects of the aged ECM in vitro	ECM from aged donors (Gullapalli et al., 2005; Williams et al., 2014; Stearns-Reider et al., 2017) Glycation crosslinking (Rodriguez-Teja et al., 2015) Enzymatic crosslinking (Levental et al., 2009)	Controversial which treatments best phenocopy aging ECM extraction from tissues can alter its mechanical properties, microstructure, and composition
Develop aging-relevant experimental readouts	Epigenetic clock (Hannum et al., 2013; Horvath, 2013) Mutational analysis (Blokzijl et al., 2016) Protein/DNA oxidation Protein aggregation Tissue-specific functional assays	Unclear which signs of aging are most important Require signs of aging that change appreciably across the span of an experiment
Long-term maintenance of organoids in a stable, growth-arrested state	Improved culture media	Relatively few published maintenance conditions
Complete modeling of whole organs and physiological systems	Improved ECM and bioreactors Co-culture with immune cells, stromal cells, and microbiome bacteria (Parrinello et al., 2005; Engevik et al., 2015; Plaks et al., 2015)	Vetting culture conditions is lengthy Few good techniques for sophisticated organoid construction
Increase throughput and reproducibility	Vascularization (Auger et al., 2013) Innervation (Workman et al., 2017) In vivo implantation (Watson et al., 2014; Takebe et al., 2015) Microfluidic access to apical/basal fluid reservoirs and fluid transport between organ systems (Verneti et al., 2017) Organ-specific ECM (Voytik-Harbin et al., 2007; Zhang et al., 2009; O'Brien et al., 2010) Defined artificial ECMs may decrease lot-to-lot variability (Gjorevski et al., 2016) Growth factor distribution within ECM gels for spatial control over growth and differentiation (Wylie et al., 2011) Cell patterning for control over initial organoid shape and composition (Nelson et al., 2008; Todhunter et al., 2015) 3D-printed gels amenable to perfusion with control over large-scale tissue structure (Kolesky et al., 2016) Morphological screening and sorting to enrich for correctly formed organoids (Arora et al., 2017)	Powerful techniques are often cumbersome and low-throughput

developed and validated, significantly accelerating human aging research and the search for therapies that will improve human health and lifespan.

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